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Food and cast analyses as a parameter of turn-over of materials by earthworms (*Lumbricus terrestris* L.)

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Summary. Earthworms (*Lumbricus terrestris* L.) were kept in glass tubes as an artificial burrow system. They were fed with maize leaves and hay, and partly artificial soil was added.
CO_2-production of the worms, food consumption, and cast production were measured. Food and casts were analysed for Ca, P, and N. *Lumbricus terrestris* utilizes only a small amount of these elements. The content of Ca, P, and N in the casts was higher than in the same amount of food.
During the passage through the gut the pH-value decreases. Ca in the gut and the cast did not influence this pH-value.

Key words: Earthworms, artificial burrows, food, cast, Co_2-production, Ca-, P, and N-turnover, pH-alteration

Introduction

More than 100 years ago Hensen (1877) and Darwin (1882) tackled the question of the significance of earthworms. Lunt & Jacobson (1944) established that the earthworm's faeces are enriched with the nutritive elements N, P, K, Ca and Mg. Most of this excrement was deposited near to the plant roots, where the increased element concentration could be absorbed directly by the roothairs (Joshi & Kelkar 1951). A mixing and disintegrating of plant and animal remains is brought about through the activity of the soil fauna, so this material is made more accessible to bacteria and fungi. The passage of organic and mineral material through the worm's gut results in the formation of more complex and stable aggregates, while a portion of the plant material is transformed into humus (Edwards et al. 1969). Of course, soil animals cannot increase the soil's nutriment content, but they do help to transform it into a more available form for plants. Sharpley & Syers (1977) investigated the chemical influence of earthworms on nutritive substances: gut enzymes act directly on the ingested organic material. A portion of the organic material is metabolised and the quantity of endmetabolics increased considerably. Also, the soil's C/N ratio is reduced in favour of N by the soil organisms. Recently Shipitalo et al. (1987) have studied the metabolic turnover of earthworms, but they only carried out food analyses. In this paper the metabolic turnover of one of soil fauna's most important representatives, the oligochaete *Lumbricus terrestris* Linnaeus is investigated. The main aim was to deduce answers to questions about metabolism by comparing chemical analyses of food and casts. In contrast to earlier studies the earthworms were kept unter artificial conditions, which allowed the supply of organic material to be controlled, so that it was possible to determine

what kind of food the experimental animals actually ingested and how the material was transformed. Actual soil with all its unknown parameters and its heterogeneous distribution of humus material is thus eliminated. In addition, it was sought to provide material for consideration of the following questions: how high is metabolic turnover and -intensity under the given conditions and by what factors are they influenced? To what extent are nutritive substances enriched in the casts of *Lumbricus terrestris*?

Material and methods

The experiments were conducted at temperatures of 8 °C and 13 °C in a dark climate chamber with 8 hours light every day. The duration of the experiment was 205 days for the 14 worms fed on maize and, owing to some initial experimental problems, 120 days for the 12 worms fed on hay. Usual, commercial hay was not accepted by them, so that it was necessary to add some inorganic substrate in the form of artificial soil (OECD 1984) which had been modified especially for these experiments: 20 g ground hay, 2 g kaolin, 138 g quartz sand, and 4 g $CaCO_3$ for each feeding box. The artificial burrows, formed after an idea of O. Kalberlah, and illustrated in Fig. 1, consisted of 50 cm lengths of corrugated glasstubes with an inside diameter of 6 mm. In order to separate these tubes for cleaning they were connected with a piece of short flexible tubing to a glass y-tube. The y-tube was inserted into the feeding box, made of polystyrene (20 × 15 × 5 cm), and anchored with a 1,5 cm thick layer of plaster. Finally, a layer of wax protected the plaster from moisture. The upper end of the corrugated glasstube was sealed with a piece of fabric. A glass vessel filled with distilled water enclosed this end to keep it moistened with water of evaporation. To prevent the possibility of the earthworms escaping, a transparent foil was bound with a strong elastic band over the feeding box. Each box accommodated one earthworm. 1 – 2 times a week faeces samples were rinsed out of the tubes with distilled water. Afterwards the samples were dried in a drying oven at 100 °C and frozen – 18 °C until they could be worked on. Samples from all the animals feeding on the same kind of food were mixed together. For purposes of comparison food samples were also collected, first before the experiment was initiated (t = 0) and again at the end of the experiment (t = end).
The ratio of food taken up to faeces egested was determined in additional experiments, also conducted at 8 °C and 13 °C. These experiments lasted between 7 and 12 days and were conducted with 7 – 13

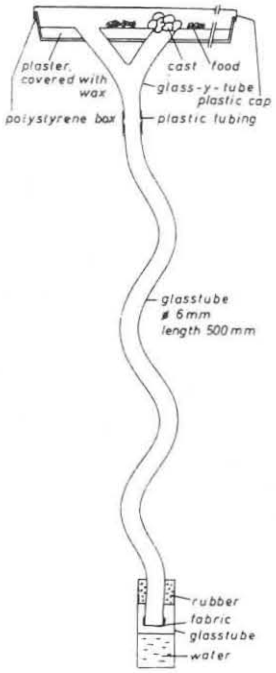

Fig. 1. Artificial burrow for *L. terrestris*

earthworms. The food (maize and hay) was dried overnight at 40 °C in a drying oven. After determining its dry weight the food were moistened and stored for 5 days so that a microbial flora could reestablish itself. Subsequently, measured amounts of food were placed in the plastic boxes (13 × 21 × 12 cm) which contained the worms. The average life weight of the worms during the experiment was ascertained. The experiment with the worms fed on hay had to be aborted.

Faeces- and food analyses

The sample material was ground with mortar and pestle until it was all passed through a 0.5 mm metal sieve before being analysed. The samples were analysed for their content of phosphorus, calcium, nitrogen and sand. In addition, pH-value of cast and food samples, the earthworm's CO_2-production and O_2-uptake were measured. The 'amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG may 1980 31.00/6' served as analytical guideline for the phosphorus determination. From a mixture of molybdic phosphoric acid and molybdic acid, only the former is reduced to molybdenum blue by the corresponding reducing agent. The amount of molybdenum blue formed is proportional the phosphorus content. The extinction of the sample solution which contained the molybdenum blue was determined photometrically (photometer: Carl Zeiss PMQ 2; sample thickness was 10 mm). After carrying-out the analysis for phosphorus the remaining main sample solution was filtered with a filterstewpan A1 and then washed with 50% concentrated HCl and dried ready for the calcium analysis. The filtrate inside the stewpan was weighed to ascertain the sand content. The digested sample material was analysed by means of AAS (Perkin Elmer, model 503) for its calcium content. As fuel gas a mixture of acetylene and nitrous oxide (N_2O) was used. The N-content was determined by the micro-method according to Kjeldahl. In this case the digestion was carried out with H_2O_2. Because of the great difficulty encountered in digesting the sample, the amount of H_2O_2 had to be increased so that it was digested within a 5–6 hour period. pH was measured with a glass electrode (WTW messtechnic pH DIGI 88). The values were ascertained first in distilled water and than in KCl solution. Data on metabolic activity were obtained by measuring CO_2 and O_2 content at intervals (during June, August and January). O_2-content was measured at defined points of time, in June, August and January. All earthworms fed on maize. For testing dependence on temperature the measurements were made at 8 °C and 13 °C in 250 ml Schottflasks sealed with a septum cap. The septa could be pierced with a syringe (Precision sampling corporation, 0.5 ml, series B). Gas samples were immediately injected into a gas chromatograph (Packard, model 419) for analysis. Each flask contained 1–5 earthworms.

Results and discussion

Influence of nutrition on the earthworms' weight

The initial average weight of the animals fed on maize amounted to 4.09 g those, fed on hay weighed a total of 2.99 g (see Fig. 2). No earthworm maintained its initial weight. The worms fed on hay showed a more or less constant average weight, while those fed on maize decreased in weight. However, the hay group was rather less active. Maize had to be replenished at times, while the initial hay feed sufficed for the duration of the experiment. In all maize feed samples a considerable portion of sand was found, probably splashed by rain on to the maize while it was in the field. Sand was enriched in the cast samples, particularly in the 8 °C maize-experiment. Between the maize faeces samples at both temperatures there were only negligible differences, which agreed with the food samples.
Sand content of the hay food and corresponding cast samples differed a little but may be considered as unchanged (see Fig. 3). The values of all following analyses refer to their organic content which was calculated previously. For a better comparison they were converted to 1 g analysed organic material. Food without sand particles was not accepted by the earthworms. On account of its small particle size, the earthworms were not able to select between the particles of hay-food and the particles of sand, but had to ingest the substrate indiscriminately. With the maize-food, on the other hand, owing to its far greater particle size, the animals were in a position to choose between inert and nutritative particles of substrate. From the enrichment of sand in the animals faeces one can conclude that

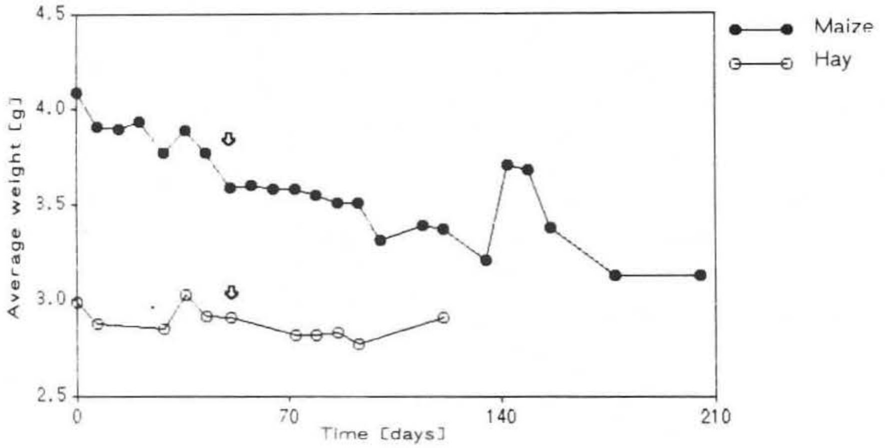

Fig. 2. Changes of *L. terrestris* life weight against time at 8 °C and 13 °C. Arrows indicate date of temperature elevation

these inorganic particles are an essential part of *L. terrestris'* diet. A portion of the organic material will be broken down during its passage through the gut and absorbed. The consequence is a concentration of inorganic particles in the faeces. Neuhauser et al. (1980) established in feeding experiments the importance of soil to the growth of *Eisenia foetida* Savigny on horse manure or sludge. Worms weight was about a third higher after 3 months in the presence than in the absence of soil. The great significance of the sand particles apparently lies in the role they play in the gut of mechanically helping to break up the particles of food and thus increasing its surface area for attack by digestive enzymes (Syers & Springett 1983). Particle size influences the earthworms' weight, too. Maize was offered in coarse chaffed pieces, the hay in a fine ground form. Boström & Lofs-Holmin (1986) found that by reducing particle size by a factor of 5, the weight of *Allolobophora caliginosa* could be doubled. If the organic material is finely divided the greater surface area results in an increased population of bacteria, which also serve as food for the earthworms (Edwards & Lofty 1977). While the earthworms fed on maize gradually lost weight the 'hay group' maintained theirs. Satchell & Lowe (1966) established that particu-

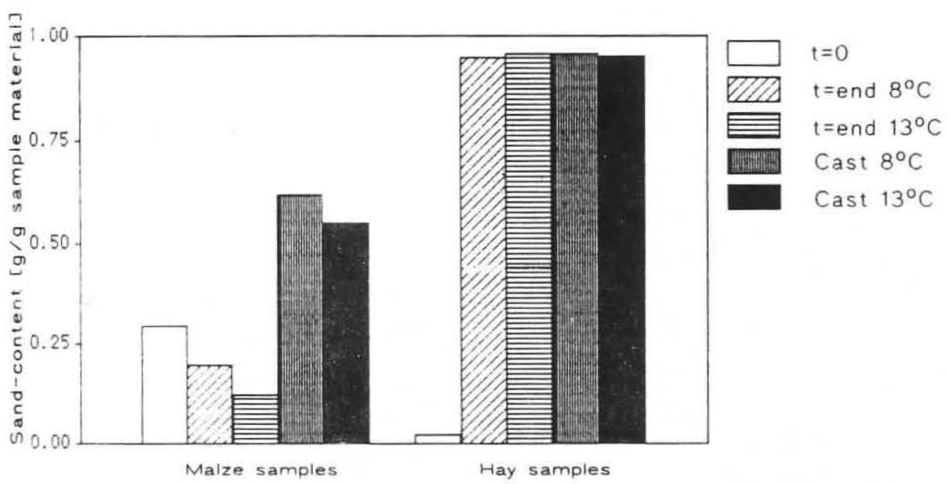

Fig. 3. Average sand content in the maize and hay samples related to 1 g sample material

larly N-containing food is preferred and results in better growth (Edwards & Lofty 1977). Maize has a nitrogen content which is about half that of hay. Sand, nitrogen content and particle size, therefore, have an essential influence on *Lumbricus terrestris* nutrition.

Food consumption and cast production

With rising temperature food intake increased, while cast production remained the same. At 8 °C the ratio of consumed food to produced faeces was 2 : 1 but at 13 °C it was 4 – 5 : 1 (see Tab. 3). The utilization of food is much better at 13 °C than at 8 °C which is confirmed by food and cast analyses and also by the gas measurements. With increasing temperature the efficiency of the enzymatic action increases, too. Within this temperature range, which corresponds to *L. terrestris'* natural environment (Graff 1971) more organic material can be broken down and absorbed. The correlation established in the food consumption/cast production experiments was about 4 – 5 : 1 at both temperatures. Comparing these results with that calculated from the calcium analyses, one notices a distinct agreement. The calculated food/cast ratio for maize was about 3 – 4 : 1. Food consumption is stubstantially influenced by temperature (Dickschen & Topp 1987), pH-value (Edwards & Lofty 1977), soil calcium content (Satchell 1955), moisture content (Graff 1971), and food palatability (Satchell 1963).

Calcium in food and faeces

$CaCO_3$ being a component of the artificial soil, the Ca-content of the food was necessarily increased. It is especially noticeable that calcium is greatly concentrated in the faeces samples, which demonstrates its little utilization by the earthworm itself. In the maize-experiments its concentration is only a little higher at 13 °C, than at 8 °C but the food-Ca : cast-Ca ratio is at 13 °C about 1 : 4 and at 8 °C about 1 : 3. Calcium consumption by the earthworm's metabolism was not taken into account (see Fig. 4). That more calcium was concentrated in the food of the 13 °C hay experiment is probably the result of incomplete mixing of all the artificial soil components. The casts of the animals fed on hay also show differences related to temperature. In the case of hay a food-Ca : cast-Ca ratio of 1 : 1.6 – 1.7 was determined. Centrary to the findings of Lunt & Jacobson (1944) a strong enrichment

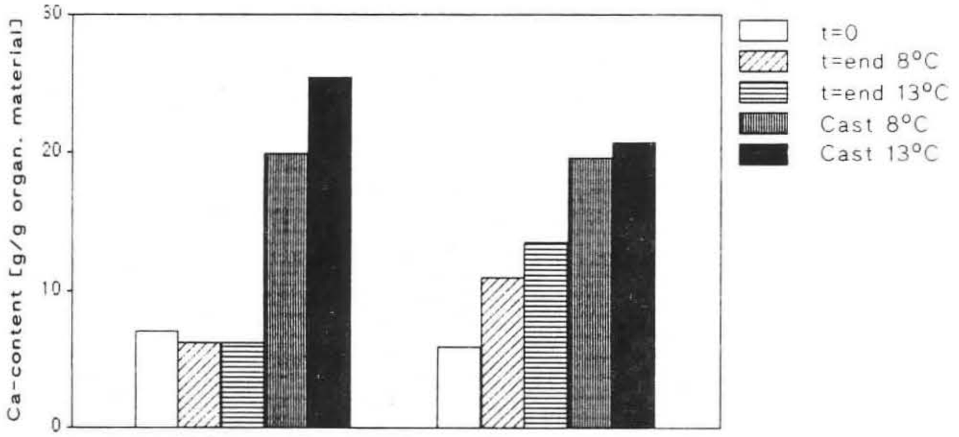

Fig. 4. Average calcium content in the maize and hay samples related to 1 g organic material

of calcium was observed in the cast of *L. terrestris*. Piearce (1972) found a concentration factor in the faeces of about 2.8, but there were strong fluctuations between different locations and species. Kale & Krishnamoorthy (1979) found that the Ca concentration in the cast varies with quality and quantity of food. A proper comparison with the data found in the literature is not possible because there the values were compared with the surrounding soil, while in these experiments values are compared with the initial Ca-content of the organic material. The concentration of the calcium in the earthworm's cast is the highest of all the elements analysed. The calcium in the faeces only originated from the food thus making an estimation of direct element turnover possible. In the maize experiment a food consumption/cast production ratio of about 3–4 : 1 was determined, depending on temperature, while in the hay experiment a ratio about 1.6–1.7 : 1 was found. This theoretically ascertained turnover allows us to make statements concerning the utilization of the elements by the earthworm.

Phosphorus in food and faeces

The faeces samples of the maize fed earthworms showed a strong concentration of phosphorus, whereby it is less pronounced at 8 °C. At 8 °C the concentration of phosphorus in the castings is almost double that in the food, while at 13 °C it is three fold. The phosphorus concentration in the hay is higher than in the maize. The hay food samples are characterized by a continuous rise in the concentration of phosphorus. At 13 °C phosphorus was enriched in the faeces, but not at 8 °C. Phosphorus, like nitrogen, is a limiting growth factor of plants. A great deal of data in the literature provide confirmation that phosphorus in a form available to plants is enriched in earthworm casts (Graff 1970; Lee 1985; Scheu 1987). The concentration in the casts of *L. terrestris* increases with temperature and corresponds to increasing enzymatic activity and metabolic rate. While the values for the maize-feed samples remain fairly constant, the hay-food samples show considerable fluctuations. Perhaps one of the artificial soil components contained phosphorus compounds and mixing was incomplete. If one compares the final food sample (hay) with the cast sample in the 8 °C experiment, no significant enrichment of phosphorus has taken place.

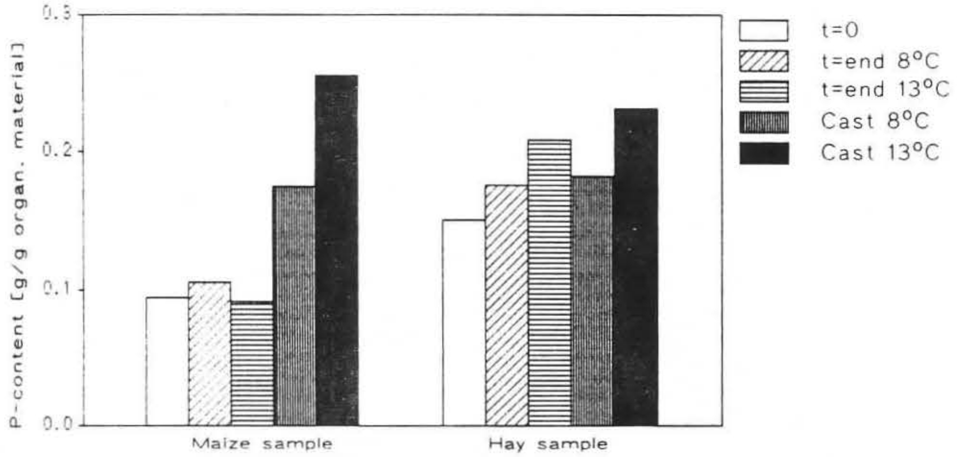

Fig. 5. Average phosphorus content in the maize and hay samples related to 1 g organic material

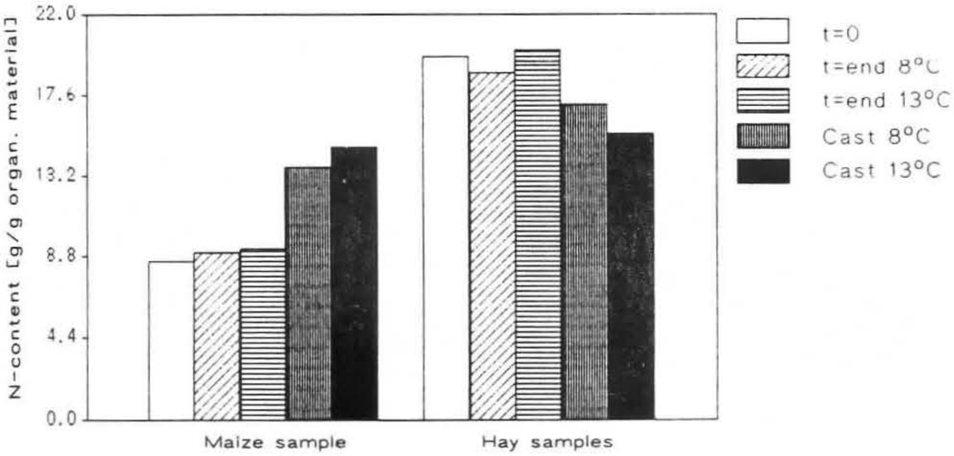

Fig. 6. Average nitrogen content in the maize and hay samples related to 1 g organic material

Nitrogen content and excretion

The faeces of the experimental animals fed on maize were enriched with nitrogen. The highest concentration was found at 13 °C. The tendency is similar but not marked as for calcium and phosphorus.

The hay's nitrogen content was more than double that found in the maize. In contrast to the maize, hay did not produce any nitrogen increase in the cast. The nitrogen content in the cast had the smallest values. Only nitrogen excreted in the casts was determined. It was not possible to determine how much nitrogen left the body with the mucous secretion or urine. That is why the ascertained N-values are always lower than in reality. The amount of absorbed nitrogen exceeded metabolic requirements by far. At higher concentrations nitrogen compounds are toxic and the body has the tendency to eliminate them (Tillinghast 1967). The calcium concentration in the casts is used as a basis for all calculations of metabolic turnover. The N-content calculated from the calcium ratio differs from that actually measured. This difference represents anabolic nitrogen and the nitrogen which is lost in urine and mucous secretion. This explains the lower than 3 fold nitrogen ratio in the casts of those earthworms fed on maize.

If one calculated the theoretical values for the hay cast samples based on calcium analyses, under the same conditions as for maize, an enrichment of nitrogen in the casts of about 1.6 – 1.7 times is obtained. Subtracting from this theoretical value the difference, which for maize was counted as non-determined, one obtained values which are very close to those actually found and which are below those in the initial food. But why the nitrogen of the hay cast sample decreased with a rise in temperature in contrast to the maize samples can not be conclusively explained. Because so much excreted nitrogen is found in the casts, *L. terrestris* must be considered a poor N-utilizer.

pH-values of the samples of food and faeces

The final food sample in the 8 °C maize-experiment could not be analysed because all of the sample material had been consumed (see arrow in Table 1). The pH-value of the food sample t = 0 was acidic at 5.6 measured in distilled water, and at 5.3 measured in KCl. The values increased during the experiment but after passage through the gut the pH-value decreased (see Tab. 1).

Table 1. pH-value of food and cast samples measured in distilled water and in KCl. (The arrow marks the final food sample of the 8 °C experiment which could not be analysed because all the sample material was consumed. M = maize, H = hay)

Samples	pH-value [aqua dest.]	pH-value [KCl]
M t = 0	5.6	5.3
M t = end 8 °C	←	←
M t = end 13 °C	5.8	5.7
M cast 8 °C	5.4	5.1
M cast 13 °C	5.5	5.2
H t = 0	5.8	5.8
H t = end 8 °C	7.2	7.8
H t = end 13 °C	7.7	7.2
H cast 8 °C	6.2	6.8
H cast 13 °C	6.1	6.8

The large increase in pH-value of the hay was due to the $CaCO_3$ content of the artificial soil. The further rise in the final sample of the 13 °C experiment is the result of incomplete mixing. The pH-value of the faeces of the earthworms fed on hay was also lower than the food. The pH-value of the soil influences the earthworm's activity and abundance (Springett & Syers 1983). Along with soil and organic material organic acids are also ingested (Laverack 1963), which may result in a drop in pH. Earthworms als secrete urine and an alkaline slime (Lee 1985). These compounds contribute to a higher pH-rise of the food, particularly when the earthworms were in the feeding boxes a long time. On the other hand *L. terrestris*' casts show a pH-value which lies clearly below that of the food. Poboszny (1975) ascertained a pH-value in the casts of some earthworm species which was closer to neutrality than was the surrounding soil. Peters (1984) could not find any difference in pH between soil and cast. The intestinal pH-value of *L. terrestris* lies between 6.2 and 6.7 (Laverack 1963). The maize experiments did not support this statement since although, the surrounding substrate was acidic, the casts' pH-value was lower still. Needham (1957) reported that when *L. terrestris* und *E. foetida* were well fed, in contrast to unfed animals of the same species, they produced higher amounts of titriable acids. Laverack (1963) established that in cast samples with low pH-values $CaCO_3$-concretions were found which did not affect the pH-value. Therefore, the strong enrichment of calcium in the casts of the earthworms fed on maize does not contradict the pH-values found within the casts. That there is no influence on the pH of the surrounding substrate by the Ca in the gut and faeces respectively was recently shown by Schrader (1991).

Gas measurements and respiration

Before the experiments were begun all animals were fed on the same food (maize) to exclude any differing effects of food on respiration. A linear relationship between O_2-consumption and time was found, illustrated in Fig. 7. O_2-consumption in the separate vessels fluctuated at both experimental temperatures. At 13 °C a higher average O_2-consumption was recorded but not in all the vessels. The data collected at 13 °C in August are on average nearly identical with the average data collected at 8 °C in June and January. There is also a linear dependancy between CO_2-production and time which is seen in figure 8. At 13 °C the earthworms produce more CO_2 than at 8 °C. CO_2-production in June is at both temperatures higher than during the other measuring periods (see Fig. 8).
The June data for both temperatures are one average nearly identical in respect to CO_2-production and O_2-consumption. In contrast, average CO_2-production at 8 °C in

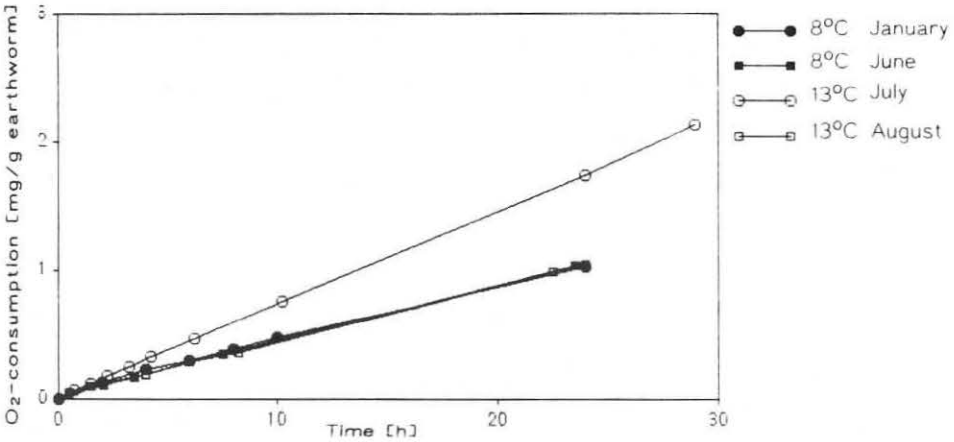

Fig. 7. Average O_2-consumption of *L. terrestris* fed on maize against of time at 8 °C and 13 °C. The single straight lines applied to the average values of all the Schottflasks at one define point of time

January is only half the corresponding O_2-consumption while in August at 13 °C it was a little higher than O_2-consumption. Comparing the data for both temperatures in June, one can see that a temperature increase of 5 °C results in a 1.8 fold increase in O_2-consumption, while the increase in CO_2-production was 1.6 fold. A comparison of the other data is not meaningful because of the seasonal fluctuations. The mean value of all the O_2-consumption data and all CO_2-production data at 13 °C are higher than the mean values at 8 °C. While O_2-consumption at 8 °C in January is higher than in June the opposite is true in regard to CO_2-production. At 8 °C mean CO_2-production is lower than O_2-consumption, while at 13 °C it is higher.

All respiratory quotients (RQ) are identical within each single month although the O_2- and CO_2-data differ strongly. When temperature increases O_2-consumption and CO_2-production increase proportionally and the RQ remains the same. The RQ values within a single month fluctuate only a little, but the fluctuations between different months are considerable.

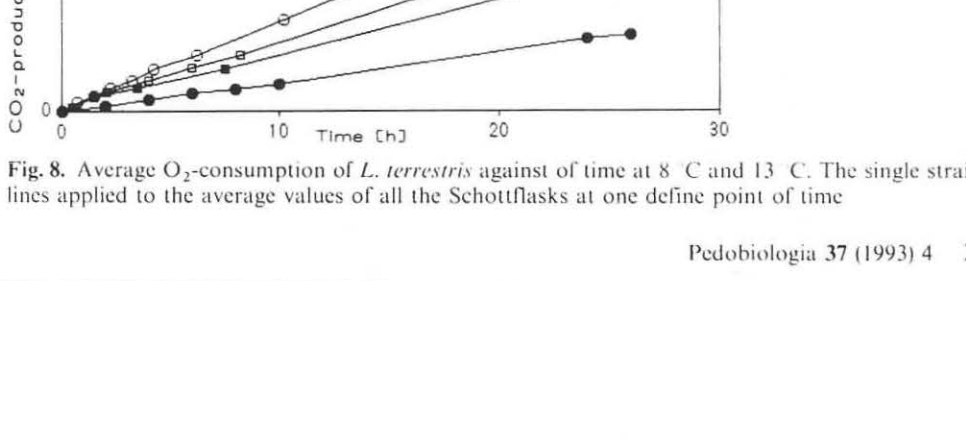

Fig. 8. Average O_2-consumption of *L. terrestris* against of time at 8 °C and 13 °C. The single straight lines applied to the average values of all the Schottflasks at one define point of time

Table 2. Average RQ of the earthworms within the Schottflasks

8 °C-experiment	RQ	13 °C-experiment	RQ
June:	1.1	June:	1,8
	8.9		8.9
	–		8.9
January:	8.5	August:	1.3
	8.5		1.1
	8.5		1.1
	8.5		1.4
	8.5		1.3
	8.5		–

As in other poikilothermic animals, temperature influences the respiration rate of earthworms. The CO_2- and O_2-data for the two temperatures are not identical. Two reasons are the different body surface and weight of the earthworms, and the seasonal fluctuation. At 8 °C and 13 °C the June values of CO_2 production are the highest. The CO_2 values for 4 different months gave 4 straight lines with different gradients. The causes are again seasonal fluctuations. In regard to the RQ it is seen in table 2 that there are unimportant fluctuations within any single month but large fluctuations between months. Bolton & Phillipson (1976) found that the RQ undergoes seasonal fluctuations at the inter- and intraspecific level. Under field conditions the following RQ's for *L. castaneus* were found: in spring 0.52, in summer 0.53, in autumn 0.58 and in winter 0.7. The tendency is not identical with the values found for *L. terrestris*, but provides an explanation for the varying RQ's. It is possible that the animal's physiology is subject to seasonal changes in connection with the formation of storage substances. It is also conceivable that here an endogenic rhythm finds expression, despite the constant laboratory conditions. Another explanation is given by Doeksen (1968) who found that a daily rhythm of activity and O_2-consumption are subject to a lunar periodicity.

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Table 3. Food consumption and cast production of the animals fed on maize at 8 °C and 13 °C

Temp.	food consumption [dry weight mg/g earthworm/day]	cast production [dry weight mg/g earthworm/day]	ratio food : cast
8 °C	25.8	13.1	2 : 1
8 °C	12.6	6.5	2 : 1
13 °C	37.1	7.3	5 : 1
13 °C	64.1	16.9	4 : 1

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